



**XVIII
CONGRESSO**
DA SOCIEDADE PORTUGUESA
DE ETOLOGIA - SPE 2021



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Abstracts

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ABOUT SPE

The Portuguese Society of Ethology was founded in 1987 and aims to promote the study of animal behavior in Portugal, considering its various aspects. Society activities include the publication of the scientific magazine Acta Ethologica, the organization of a National Congress and other scientific meetings, the promotion of international relations with similar societies and the dissemination of Ethology at the level of secondary education.

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Plenaries

Plenary 1 – Dr. Lilian Manica (Universidade Federal do Paraná, Brazil)

Courtship in a neotropical manakin: a synchronized and coordinated spectacle

In this talk I will introduce the audience to the marvelous Swallow-tailed Manakin, an endemic Atlantic Rainforest bird species that perform a highly complex cooperative display to females. I will show some of the main findings of our lab's research in the past years, including natural history, ecology, bioacoustics, and physics of the coordinated display which may involve up to eight males.

Plenary 2 – Dr. Lee Alan Dugatkin (University of Louisville, USA)

Mutual aid: Peter Kropotkin's adventures in science and politics

Peter Kropotkin was one of the great thinkers of the late 19th and early 20th centuries. He wrote incessantly, penning books on evolution, behavior and natural history, ethics, anarchism, socialism and communism, the coming industrial revolution in the East, penal systems, The French Revolution and much more. Though seemingly disparate topics, a common thread--Kropotkin's biological law of mutual aid, which guided all living things from microorganisms to nonhuman animals to humans--tied all of these works together. What Kropotkin then called mutual aid, we now call cooperation

Kropotkin read Darwin when he was a teenager in Moscow. He came to see that evolutionary principles explained the diversity and history of life. Over time he came to argue that natural selection was the driving force that shaped life, but that Darwin's ideas had been perverted and misrepresented by {primarily} British scientists. Natural selection, Kropotkin proposed, generally led to mutual aid, not competition, among individuals

Plenary 3 – Dr. Paulo Gama Mota (Universidade de Coimbra, Portugal)

Bird communication: the signaling role of sexual and social selection



Round Table

Social Isolation

Speaker 1 – Dr Culum Brown (Macquarie University, Australia)

Social isolation in fishes

Fishes are renowned for their spectacular schooling behaviour which serves to remind us that many of them are highly social animals. In fact, their social intelligence rivals most terrestrial vertebrates. Their social structure ranges from highly aggressive, territorial species right the way through to species with complex family groups that collectively raise young. Group size is dependent on cost/benefit computations and for many species there is a strong incentive to remain in close proximity to shoal mates, while for others they prefer to remain alone. Based on this, one might predict that the effects of social isolation will vary considerably across species and thus fishes offer a great opportunity for comparative studies. Intriguingly, however, even among social species the data suggests that brief periods of social isolation can actually be less stressful than remaining in social groups. This indicates that while socially is clearly beneficial it can also be hard work! Here I will provide a brief overview of the impacts of social isolation on fishes including stress, anxiety & depression. The primary take-home message is that the behavioural and physiological impacts of social isolation are very similar to those seen in humans and the rest of the vertebrate kingdom.

Speaker 2 – Dr. Caio Maximino Oliveira (Universidade Federal do Sul e Sudeste do Pará, Brazil)

Mental distress, loneliness, and coping during the COVID-19 pandemic

Social isolation has negative impacts on physical and mental health and longevity across animals, including human and non-human ones. Across the world, as an attempt to reduce contagion by the SARS-CoV-2 coronavirus, communities relied on isolation as a strategy, but not without consequences. As a social species, humans were highly affected by the pandemic. In Brazil, the COVID-19 pandemic proposed "infernal alternatives" (*a la* Stengers) between starving and suffering of loneliness, and dying of COVID-19 and/or being responsible for the death of loved ones. Drawing on studies across the world as well as our own research, the consequences of social isolation during the COVID-19 pandemic are discussed. Psychological factors - including feelings of loneliness, entrapment, and specific coping strategies - are shown to mediate the effects of social isolation on psychological distress.



Speaker 3 – Dr. Jorge Gato (Universidade do Porto, Portugal)

Effects of the COVID-19 pandemic on the mental health of LGBTQ+ young adults: the role of social isolation and other variable

Across the world people have seen their lives interrupted by the COVID-19 pandemic. Using an online survey, we explored how the psychosocial effects of the pandemic affected the mental health of LGBTQ+ young adults who were confined with their parents during the lockdown period ($N = 1,934$), from six countries: Portugal, UK, Italy, Brazil, Chile, and Sweden. Depression and anxiety were higher among participants who were younger, not working, living in Europe and who reported feeling more emotionally affected by the pandemic, uncomfortable at home, or isolated from non-LGBTQ friends. Not attending higher education predicted depression while not being totally confined at home, residing habitually with parents, and fearing more future infection predicted anxiety. LGBTQ+ community groups, as well as health and educational services should remain particularly attentive to the needs of LGBTQ+ young adults during health crises.

Speaker 4 – Dr. Nathan Bailey (University of St Andrews, UK)

Social isolation as an extreme environment: evolutionary causes and consequences

Social isolation is commonly studied in the context of its negative impacts on health, longevity, and other fitness traits within a single generation. Less is known about its cross-generational evolutionary consequences. I will provide an overview of these: from releasing cryptic genetic variation and altering adaptation rates, to shaping patterns of diversification. With observations from a rapidly evolving field cricket system in which the dominant communication signal, song, has been evolutionarily lost, I illustrate how evolutionary dynamics arising from social isolation could influence the likelihood and rate of adaptive evolution. I advocate for treating social isolation not as a baseline control condition in experimental work, but as a focal treatment condition in its own right.



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Posters presentations



Revealing complex relationships between individuals' phenotype variation and social signalling in a range of life-history strategies

Caterina Funghi ^{1,2}, Ana V. Leitão^{2,3}, Paulo Mota ²

¹Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) Istituto Superiore per la Ricerca Ambientale, Via Cà Fornacetta 9, 40064 Ozzano dell'Emilia (BO), Italy ² Research Center in Biodiversity and Genetic Resources (CIBIO), Coimbra / Vairao, Portugal. ³ School of BioSciences, University of Melbourne, VIC, Australia

In social living animals there are two ways an individual may find food: joining a conspecific using social information (scrounger), and/or using personal knowledge (producer). These tactics have been linked to different life-history strategies. Hierarchical access to foraging patches may constrain the use of the producer-scrounger tactics and select for colour ornaments to be badge of -status. In sexual dimorphic species, males and females present a different life-history strategies and often females do not display the badge-of-status and are subordinates. Here, we studied the phenotypic variation of males and females European serins, *Serinus serinus*, measuring colouration, foraging ability in solitary and with a companion, and defining them within a boldness-shyness axis. Quantifying foraging performance in solitary context showed that more colourful individuals were faster to find food, regardless of their sex. Bolder males were faster finding food than shier ones. The opposite was found in females. With a companion, less colourful males and more colourful females preferred the producer strategy. Additionally, males, but not females, with a bolder companion preferred the producer strategy. Overall, these results provide an unprecedented view in the serins' foraging context, clarifying how sex and foraging ability mirror different life-history strategies and concur to explain phenotype variability.



The Role of Hyperpolarization-activated Cyclic Nucleotide-Gated Channels, Indirectly Stimulated by Nitric Oxide, in the Maintenance of Sensitization of Anxiety-Type Responses in a Zebrafish (*Danio rerio* Hamilton 1822) Post-Traumatic Stress Model.

Amanda Gabriele Oliveira Xavier¹, Larissa Mota de Freitas¹, Sara Letícia de Paula Torres¹, Carlos Eduardo dos Santos Almeida¹, Caio Maximino², Monica Gomes Lima Maximino¹

¹Laboratório de NeuroFarmacologia e Biofísica, Departamento de Morfologia e Ciências Fisiológicas – CCBS, Universidade do Estado do Pará – Campus VIII, Marabá, Pará, Brasil

²Laboratório de Neurociências e Comportamento “Frederico Guilherme Graeff”, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Marabá, Pará, Brasil.

Time-dependent sensitization (TDS) is a model to study Post Traumatic Stress Disorder (PTSD). We have previously demonstrated the participation of NO in TDS. Here we investigate the role of hyperpolarization-activated cyclic nucleotide-gated channels (HCNs) in the regulation of behavioral responses in TDS. 144 Adult longfin zebrafish were treated with either vehicle, a NO substrate (L-arginine; 100 mg/kg), or a HCN inhibitor (Ivabradine; 10 mg/kg) 30 min or 90 min after exposure to water or alarm substance (AS). After 24h, the behavior of the animals was evaluated in the light-dark preference test. Exposure to the stressor sensitized dark preference [30min: $F(1,58) = 37.48$, $p < 0.0001$; 90min: $F(1,58) = 14.56$, $p = 0.0003$] and risk assessment [30min: $F(1,58) = 29.88$, $p < 0.0001$; 90min: $F(1,59) = 29.49$, $p < 0.0001$]. Ivabradine blocked risk assessment sensitization with 90 min treatment after exposure [$F(2, 59) = 10.63$, $P = 0.0001$, interaction effect]. L-arginine potentiated the sensitization on risk assessment [90 min: $F(2,58) = 9.451$, $p = 0.0003$; interaction effects]. We conclude that NO participates in the modulation of stress sensitization in zebrafish, and HCN channels have an important role in the 90 min time window.

Keywords: Zebrafish, Post Traumatic Stress Disorder, CGN.

Apoio financeiro: CNPq-Brazil.



Impact of anthropogenic noise on the behavior of meagre, *Argyrosomus regius*, juveniles

Rita Trabelo², André B. Matos¹; Manuel Vieira^{1,3}; Ana Faria¹; Paulo J. Fonseca³; M. Clara P. Amorim^{1,2}

¹MARE – Marine and Environmental Sciences Centre, ISPA-Instituto Universitário, Lisbon, Portugal.

²Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

³Departamento de Biologia Animal and cE3c Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

Meagre (*Argyrosomus regius*) is vocal fish that uses the Tagus estuary as reproduction grounds, an area with intense marine traffic. Here, we studied the effects of boat noise exposure on the behaviour of meagre juveniles, namely approach and pursuit. We exposed juvenile groups to either noise (boat noise playback) or control treatments (coils producing a similar electric field to the speakers) and recorded their pre- and post-feeding behaviour after 2 and 7 days of exposure. The ratio of approaches between post- and prefeed periods was always >1 in both groups, suggesting feeding arousal or increased food search. Interestingly, approach ratio decreased from day 2 to day 7 in the noise group but increased in the control suggesting that these increase in arousal or search behaviour with feeding is being affected by prolonged noise exposure. Pursuit was not affected. Whether these results have implications later in their development remains to be investigated. Also, tested fish were raised in an aquaculture facility, i. e. in much noisier environment than the natural one. Further studies with wild meagre could help shed some light on this issue and will give us a better understanding of how anthropogenic noise can impact meagre early in life.



Choosing the best mate: Female and male plumage color is linked to parental quality, pairing, and extrapair mating in a tropical passerine

Ana V. Leitão^{1*}, Michelle L. Hall^{1,2,3}, Raoul A. Mulder¹

¹School of BioSciences, The University of Melbourne, Parkville, Melbourne, VIC, Australia

²Bush Heritage Australia, Melbourne, VIC, Australia

³School of Biological Sciences, The University of Western Australia, Crawley, WA, Australia

Sexual selection has been proposed to drive the evolution of elaborate phenotypic traits in males, which often confer success in competition or mating. Female birds, like males, may display bright plumage, although studies examining how selection acts on both sexes are scarce. Using a field study, we investigated whether plumage ornamentation is sexually selected in females and male's lovely fairy-wren *Malurus amabilis*, a cooperatively breeding songbird. We found that female and male plumage color was correlated with parental quality but not with individual quality or survival. We also found evidence of positive assortative mating based on plumage color. Microsatellite analyses of paternity indicated that the lovely fairy-wren has high levels of extrapair paternity (EPP), with 53% of offspring resulting from extrapair (EP) mating. Female and male plumage color did not predict reproductive success or the proportion of EP offspring in their own nest, but less colorful males obtained higher EPP when paired with more colorful females and gained overall higher total paternity (own nest and other nests). We argue that plumage color may be under sex-specific selection, highlighting the importance of looking at both sexes in studies of sexual selection and ornament evolution.



Oxybenzone impairs short-term memory in adult zebrafish

Bruna Beatriz Ferreira, Ana Luisa Pires Moreira, Ana Carolina Luchiari

Department of Physiology and Behavior, Biosciences Center, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil

The present study aims to evaluate the effects of Oxybenzone, a chemical UV filter that is widely used by the cosmetic industry and has been considered an emerging contaminant in aquatic ecosystems. We tested the effects of chronic oxybenzone exposure on shortterm memory in zebrafish. Fish were exposed to different concentrations of Oxybenzone (10, 100 and 1000 $\mu\text{g L}^{-1}$) for 15 days and then tested in a T-maze with cues. Each arm of the maze showed geometric forms on the walls that served to differentiate them. Fish were individually allowed to explore 2 arms of the maze for 5 min (training), then a 1- hour interval followed, and the fish returned to explore the maze for another 5 min when it could access the 3rd arm (probe). Zebrafish was expected to explore the novel arm more than the others on the test as it indicates memory of the training phase. Fish treated with oxybenzone spent less time in the novel arm, presented higher latency to enter the novel arm, and showed reduced locomotion. These results suggest that oxybenzone affects the fish performance in the task, jeopardizing the animals' ability to navigate, perceive environmental signals, and retain information.



Mate-choice copying in fruit flies is regulated by appetitive dopaminergic neurons

Joana Marcos¹, Susana A. M. Varela^{1,2*}, Maria L. Vasconcelos³, Rui F. Oliveira^{1,2,3*}

¹Instituto Gulbenkian de Ciência, Oeiras, Portugal

²ISPA-Istituto Universitário

³Champalimaud Centre for the Unknown, Lisboa, Portugal

*PI and co-PI of the Social Fly Project

Dopamine is a neuromodulator that controls the formation of appetitive and aversive memories during the associative learning process. Mate-choice copying (MCC) is a type of associative learning during which females observe the mating interactions of other females and copy their choices. MCC is known to be regulated by dopamine but is unclear whether it depends on appetitive or aversive memories, as females can learn to copy preferences (appetitive memory) or rejections (aversive memory). As motivation is necessary for reproduction and appetitive learning increases motivation, MCC probably involves the formation of appetitive memories. To find out, we studied the role of appetitive (PAM) and aversive (PPL1) dopaminergic neurons in modulating MCC, in *Drosophila melanogaster*. We used transgenic lines to silence dopaminergic signalling in the PAM and PPL1 clusters. We compared the behaviour of transgenic with control flies. We found that PAM flies were incapable of MCC, unlike control flies. PPL1 flies also failed to learn but learned better than PAMs (although the sample size is small). This confirms that the formation of appetitive memories during MCC is certainly an adaptive mechanism. For this reason, there may be a smaller contribution from aversive memories, however, our study leaves this hypothesis still open.



Differences in early alcohol exposure effects in three zebrafish (*Danio rerio*) populations

Jackson Nazareno Gomes de Lima, Thais Agues-Barbosa, Ana Carolina Luchiari

Department of Physiology & Behavior, Universidade Federal do Rio Grande do Norte, Rio Grande do Norte, Brazil

Zebrafish are widely used in alcohol effect studies, including the consequences of embryonic alcohol exposure, leading to fetal alcohol spectrum disorders - FASD. FASD may cause cognitive and behavioral impairments, depending on the amount of alcohol, frequency of exposure, embryo development stage, and genetic variations. In this study, we tested the effects of embryonic alcohol exposure on the behavioral responses of three zebrafish populations: AB, TU, and WT. Eggs (24hpf) were exposed to 0%, 0.5% or 1.0% alcohol for 2h. At 6dpf, fish larvae were tested for locomotor and anxiety-like responses in a novel tank test. Both AB and WT larvae previously exposed to 1.0% alcohol showed increased locomotion (hyperactivity), while TU larvae exhibited diminished motor response (hypolocomotion). The three populations treated with alcohol showed anxiouslike behavior, observed by decreased time at the edge of the tank. Our results indicate population-dependent effects of embryonic alcohol exposure observed at the larval stage. While FASD is difficult to diagnose and lack proper treatments, our study presents a common anxiety-like behavioral pattern between populations, but hyperactivity present in only some populations. These findings contribute to a better understand of FASD and suggest specific populational variations.

Keywords: Anxiety-like; Motor response; WT, TU, AB



Fish sound diversity in a tropical coral reef: the Mozambique Island

Marina Puebla-Aparicio^{1*}, Claudia Ascencio-Elizondo^{1*}, Manuel Vieira^{2,3,4}, Ricardo Duarte⁵, M. Clara P. Amorim^{2,4} & Paulo J. Fonseca^{2,3}

¹International Masters in Biological Resources, Gent University, Belgium

²Faculdade de Ciências, Universidade de Lisboa, Portugal

³cE3c—Centre for Ecology, Evolution and Environmental Changes, Lisbon, Portugal.

⁴MARE – Marine and Environmental Sciences Centre, ISPA-Instituto Universitário, Lisboa, Portugal.

⁵Universidade Eduardo Mondlane, Maputo, Mozambique and CAIRIM - Centro de Arqueologia e Investigação em Recursos da Ilha de Moçambique

Coral reefs are biodiversity hotspots in urgent need of protection in most areas of the tropical belt due to increasing anthropogenic pressures and overall climate change. Evaluating fish biodiversity is key to assess the ecological state of the reefs. The present study aims to take advantage of fish vocalizations to evaluate the state of the fish community of the Mozambique Island reef, which is under considerable human pressure. Fish sound diversity and abundance were assessed using Passive Acoustic Monitoring (PAM). Recording devices were deployed 33 stations on March-April 2017 and 2018, recording continuously for ca. 24 h (44.1 kHz, 16 bit). A total 298,327 fish sound occurrences of 47 fish sound types were recognized, from which 28 (with a range of 10-20 sound sequences) were analyzed and characterized. A systematized catalog of fish sound types was prepared. In addition, the acoustic recordings were examined for time and frequency partitioning of these sound types. Such knowledge may pave the way to assess changes in reef fish biodiversity, with implications for the management of this and other reef areas where anthropogenic pressure is likewise increasing.



Impact of pool size of forced swim test in the detection of the antidepressant-like effect of chronic chrysin treatment

León Jesús German-Ponciano^a, Abigail Romero-Hernández^b, Lízbeth Bautista Castañeda^b, Gilberto Uriel Rosas-Sánchez^a, Adolfo Sánchez-Flores^{c,d}, Emma Virginia Herrera-Huerta^{c,d}

^a Instituto de Neuroetología. Universidad Veracruzana. Xalapa, Veracruz (México)

^b Programa de Químico Farmacéutico Biólogo. Universidad Veracruzana. Orizaba, Veracruz (México)

^c Facultad de Ciencias Químicas. Universidad Veracruzana. Orizaba, Veracruz (México)

^d Cuerpo Académico “Farmacología Clínica y Molecular”, Facultad de Ciencias Químicas. Universidad Veracruzana. Orizaba, Veracruz (México)

The forced swim test is the most used animal model to identify potential antidepressant substances; however, methodological conditions can interfere in detection of novel antidepressant drugs such as the flavonoid chrysin, which has demonstrated antidepressant like effect. The aim of this study was to determinate the influence of pool size in the identification of the possible antidepressant-like effect of chronic chrysin treatment. Forty eight male Wistar rats were assigned in two groups: one in a rectangular pool (30 x 50 x 60 cm) and another in a square pool (29 x 26 x 40 cm), divided in turn into three subgroups: vehicle (DMSO 10%), chrysin (5 mg/kg) and fluoxetine (1 mg/kg) which were administered during 28 consecutive days. Before treatments, rats were submitted to pretests of locomotor activity test (LAT; 5 min) and forced swim test (FST; 15 min). On 28th day of treatment, rats were submitted to the test of LAT (5 min) and FST (5 min). The results showed in the FST that chrysin 5 mg/kg and fluoxetine 1 mg/kg reduced the immobility and increase the swim time only in the rectangular pool, respect to the vehicle group. In another hand, climbing was grater in the square pool compared to the rectangular pool. The were no significant differences in crossings in the LAT. In conclusion, only with the rectangular pool was possible to identify the antidepressant-like effect induced by the chronic treatment with chrysin. While, in the square pool climbing was greater than rectangular pool, possibly due to the pool conditions. The above must be considered in future research in order to refine and improved the predictive validity of this model.



Effect of tactile stimulation on social interactions and stress in a compressed bodied cichlid

Lethicia Vian^{1*}, Ana Carolina dos Santos Gauy^{1,2}, Eliane Gonçalves-de-Freitas^{1,2}

¹Departamento de Ciências Biológicas, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista - UNESP, Campus São José do Rio Preto, SP, Brazil,

²Centro de Aquicultura da UNESP, Campus Jaboticabal, SP, Brazil

Physical stimulation is known to reduce stress in several animals, including fish. Here we tested the effect of tactile stimuli on social interactions and stress in groups of Angelfish (*Pterophyllum scalare*). Juvenile Angelfish were gathered in threes and assigned to either the treatment with (N=15) or without (N=14) tactile stimulation. For tactile stimulation, an apparatus made of PVC with sticks bordered by silicone bristles was positioned centrally in the aquarium, which fish could swim through spontaneously. A similar apparatus without bristles was used for the control group. Fish were analyzed for 14 days, being videotaped in 10min/day sessions on the first day and subsequent even days, to quantify aggressive interactions and crossings through the apparatus. Biometrics were carried out on days 1 and 14 and blood collection for analysis of cortisol level was done on the 14th. Although fish spontaneously crossed the apparatus in both treatments, there was no difference in cortisol levels and aggressive interactions between treatments. Tactile stimulation has, however, changed the dynamics of fish's growth performance, with greater growth of the alpha fish. We concluded that tactile stimulation is not aversive to angelfish, however, it was not effective for improving fish's welfare.

Keywords: aggressiveness, angelfish, cortisol, social hierarchy



The smell of a conspecific of the opposite sex modulates the vigilance behaviour of the European mink during mating season

Lorena Ortiz-Jiménez¹, Isabel Barja^{1, 2}

¹Department of Biology, Zoology Unit, Universidad Autónoma de Madrid, Madrid, Spain.

²Biodiversity and Global Change Research Center (CIBC-UAM), Universidad Autónoma de Madrid, Spain

The European mink is a solitary carnivore that relies on chemical communication to detect signals from conspecifics. This communication is crucial during breeding season since finding potential mates with whom to reproduce depends on its effectiveness. Our study evaluated the duration of European mink vigilance behaviour when exposed to the faeces of individuals of the opposite sex and two ages groups. The results showed that minks increased their time spent on vigilance in the face of faeces of individuals of the opposite sex probably in order to assess the proximity of a conspecific to avoid a metabolically costly confrontation or, on the contrary, to confront an intruder in defence of the territory. The presence of faeces from an adult female elicited an increase on vigilance behaviour duration of adult males with respect to the faeces of a subadult female probably due to a male predisposition in choosing a sexually mature mate (detected by volatile compounds in faeces). In *ex situ* conservation programs, the choice of mates is made under genetic criteria to avoid inbreeding, so it may be of interest to previously stimulate males with female scents before bringing them together for copulation.



The influence of short-term captivity on the behaviour and behavioural stress of wild-caught wood mice (*Apodemus sylvaticus*)

Maílís Carrilho*, Guilherme Aparício*, Maria da Luz Mathias*, Sophie von Merten*, Joaquim Tapisso*

*CESAM – Centro de Estudos do Ambiente e do Mar, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Several studies investigated the effects of medium-long term captivity of animals from wild populations related with rehabilitation, conservation programs or reintroductions. However, the consequences of short-term captivity are much less approach, particularly in small-sized animals kept for experimental purposes. With this study, we aim to assess if there is a relation between the behaviour of wood mice and their behavioural stress levels during short-term captivity. We hypothesize that animals adjust to captivity after a certain amount of time and that this is reflected in both behaviour and stress levels. To test our predictions, we hold animals in captivity for four weeks and measure behaviour and stress levels once per week. We perform behavioural tests (open-field test and shelter-exit test) to assess the activity and boldness of the captive animals. To measure the behavioural stress levels, we film the animals' behaviour inside their home cages during the night to observe possible stereotypic behaviours and evaluate the nest quality. Our first results show that the tested animals seem to get habituated, both behaviourally and considering their stress-levels, to the captivity conditions over the time. Future studies are needed to know if this habituation can influence the success of the animals after releasing



Comparative analysis of song evolution by natural and sexual selection of two families of Neotropical birds

Natália Simoni Porzio¹, Paulo G. Mota²

¹Universidade de Coimbra CIBIO

²Departamento de Ciências da Vida, Universidade de Coimbra CIBIO

Songs are greatly diverse among birds, both due to ecological factors and to sexual selection. We studied two passerine families, Pipridae (suboscine) and Cardinalidae (oscine) that widely differ in song structure, ecological and life-history parameters, besides their differences regarding morphology and vocal constraints. Our objective was to determine how natural and sexual selection modulated the evolution of song structure in both families. We tested whether environmental variables affected several acoustic parameters we measured through a PGLS analysis and estimating the phylogenetic signal of each song trait. We found a strong phylogenetic signal for most of the song parameters in Cardinalidae, unlike in Pipridae. PGLS results showed that nine song parameters of Cardinalidae are constrained by ecological factors and morphological traits, while six are in Pipridae. Body length was related with frequency parameters only in Cardinalidae, but it related negatively with entropy in both families (smaller birds have more complex songs). Nest height was an important predictor of song duration and complexity in Pipridae. In Cardinalidae habitat cover was negatively related with entropy, and migratory species have larger repertoires, as expected if under sexual selection. We discuss our results by comparing two passerine families that are differently constrained in singing ability.



Early social development of wild yellow breasted capuchin monkey

Nayara Teles; Delval, I.; Faverin, E.; Izar, P.

University of Sao Paulo, Psychology Institute - Department of Experimental Psychology.

In this study we aimed at describing and analyzing the pattern of sociability relationship between offspring and mother along the initial development of wild infants of capuchin monkeys from Una Biological Reserve, Brazil. We addressed eight developmental points of eight infants, four females and four males: the 1st, 2nd, 3rd, 9th, 15th, 21st, 27th, and 33rd months of life. We recorded four behaviors that are crucial for the development of social relationships (1) proximity, (2) grooming, (3) carrying on the back and (4) breastfeeding. The focal individuals were the infants and observations were made with their respective mothers. In the first months of life the relationship with the mother is much closer than in the following months, when there was a decline in all the four behaviors recorded. This result probably reflects the acquisition of independence for feeding and locomotion. There was a sex difference in the frequency of the observed behaviors, namely that females had higher values than males, what can indicate differential maternal investment in the offspring.



Oxybenzone exposure decreases aggressive behavior in adult zebrafish

Paula Francinete Nascimento de Lima, Ana Luisa Pires Moreira, Ana Carolina Luchiari

Department of Physiology and Behavior, Biosciences Center, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil

The increased ultraviolet (UV) radiation on the earth's surface boosts the need for products containing UV filters. One of the most used is oxybenzone, which is indiscriminately discarded in the environment. In this study, we investigated the effects of oxybenzone on zebrafish aggressiveness. Fish were exposed to different oxybenzone concentrations (10, 100 and 1000 $\mu\text{g L}^{-1}$) for 15 days and submitted to the mirror test. The tank presents a mirror close to the lateral wall, but an opaque partition prevented fish from accessing the mirror image. Fish were individually placed in a tank where behavior was recorded for 5 min, and then it could access the mirror image for another 5 min. As zebrafish does not recognize itself in the mirror, it displays aggressive behavior toward the reflection, which signals a potential competitor. Fish exposed to oxybenzone increased the latency to approach the mirror and showed fewer mirror bites than the control group. These results suggest that oxybenzone affects threat perception and impairs the proper aggressive response in a contest/competition context. In nature, it may affect aquatic animals' performance and reduce fitness.



A test of context and sex-dependent dopaminergic effects on the behavior of a gregarious bird, the common waxbill *Estrilda astrild*

Sandra Trigo, Paulo A. Silva, Gonçalo C. Cardoso, Marta C. Soares

CIBIO/InBIO—Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

The Dopaminergic (DAergic) system has well known influences on behavioral and cognitive functions. Previous work with common waxbills reported context-specific DAergic effects that could have been due to social environment. Manipulating the dopamine D2-like receptor family (D2R) pathways had opposed effects on behavior depending on whether waxbills were tested alone or in a small cage with a mirror serving as social stimulus. Since waxbills are highly gregarious, it was hypothesized that companion might explain this context-dependence. To test the context-specific DAergic effects, we compared behavioral effects of D2R manipulation in waxbills in the same familiar environment, but either alone or with a familiar, same-sex companion. We found that D2R agonism decreased movement and feeding, similarly to previous results. However, contrary to the hypothesis of dependence on social context, we found that the behavioral effects of the D2R agonist were unchanged when waxbills were tested with a companion. The context-dependence reported earlier might thus be due to other factors. We also found a sex-specific social effect of D2R manipulation: D2R blocking tended to decrease aggression in males but to increase in females. Together with past work, our results suggest that DAergic effects on behavior involve different types of context- or sex-dependence.



Anti-predatory strategies of butterflies from two Brazilian biomes

Sofia Coradini Schirmer¹; Márcio Zikán Cardoso²; Daniel Marques de Almeida Pessoa³

¹Programa de Pós-Graduação de Psicobiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, RN, Brasil,

²Laboratório de Ecologia e Evolução de Borboletas, Departamento de Ecologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, RN, Brasil. ³Laboratório de Ecologia Sensorial, Departamento de Fisiologia e Comportamento, Centro de Biociências, Universidade Federal do Rio Grande do Norte, RN, Brasil.

Ecosystems affect the evolution of animal coloration through convergent evolution since they can influence the efficiency of anti-predatory strategies and socio-sexual signaling. Here we focus on different anti-predatory strategies displayed by butterflies from open (White Forest and Savanna) vs. closed (Tropical Forest) vegetation and evaluate if these insects show divergent wing coloration. According to the coloration pattern exhibited on the ventral surface of their wings, a total of 129 species, that occur exclusively in the White Forest/Savanna (N = 18) or in the Tropical Forest (N = 32), had their anti-predatory strategies categorized as: camouflage, transparency, aposematism or wing eyespot. We found that butterflies inhabiting open and closed vegetation display the same proportion of anti-predatory strategies. When compared the proportion of butterfly that share biomes (N = 79), we found that butterflies that occurred in both biomes are not more camouflaged than butterflies that only occurred in one biome (N = 50). Our negative results may result from the role that butterfly coloration exerts in socio-sexual signaling. Once different ecosystems have various microhabitats, which differ in the spectral composition of light, dynamic signaling in butterflies might be convergently selected for all biomes



Environmental unpredictability, status and higher education: how childhood resources are related to our degree

Tainah Porpino de Paiva Costa, Felipe Nalon Castro

Laboratory of Evolution of the Human Behavior, Graduate Program in Psychobiology, Department of Physiology, Federal University of Rio Grande do Norte, Natal, Brazil

Life history theory describes how organisms allocate their resources throughout their development. Several factors can influence the allocation of these resources, including environmental unpredictability. In humans, the unpredictability experienced in the family environment during childhood precedes a mental unpredictability model that influences the behavior of the adult individual, such as time invested in education and career decisions. This study investigated whether financial unpredictability in childhood is related to the status of the chosen higher education course. For this, 347 undergraduate students (211 women) answered a sociodemographic questionnaire, assessed the status of the undergraduate course they are partaking through the Subjective Social Status Ladder and indicated the level of financial resources scarcity experienced in childhood through the Family Unpredictability Scale in Childhood. The results indicated that students of both sexes who experienced lack of financial resources during childhood rate their courses with lower status. It was also verified that individuals with higher levels of financial insecurity belong to lower social classes. The present work suggests that the environment experienced in childhood influences the behavior of decision-making in adult life. However, more studies are needed to verify whether entry into low status courses is in fact a student preference.

Keywords: Career choice, financial resources, status.



Do infant capuchin monkeys differ in how dependent they are?

Beatriz Felício; Natalia Albuquerque; Patrícia Izar

Institute of Psychology, University of Sao Paulo, Sao Paulo, Brazil

The aim of this work was to verify the existence of inter-individual behavioral differences in the 'dependence' category of four capuchin monkey (*Sapajus libidinosus*) infants (two males and two females). The 2nd and 9th months of life were chosen for the analysis, as they represent distinct and important developmental points. To do so, videos (sampling: focal animal) of a wild group from Fazenda Boa Vista, Brazil, were used. The videos were randomized and 10 minutes of each month for each individual were coded. To assess 'dependence', we chose the behaviors: independence and dependence, defined by the spatial proximity to another individual (juvenile or adult), social engagement (behavior of approaching an adult), and energy (locomotion). The animals did show differences in the means for each behavior, but, due to the great intra-individual variation, it was not possible to identify significant differences between individuals for each variable. These results corroborate previous data that indicate low consistency for the behavior of young capuchin monkeys and suggest the need for studies of individual profiles to better understanding the behavioral tendencies in the species.

Keywords: behavioral differences, development, interindividual differences, social behavior; capuchin monkeys; neotropical primates; *Sapajus libidinosus*

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Effect of ingestion doses of combined herbicides on the *Apis mellifera* honeybee's behavior

Juliana Sartori Lunardi¹, Rodrigo Zaluski², Ricardo de Oliveira Orsi³, Percilia Cardoso Giaquinto¹

¹São Paulo State University (UNESP), Institute of Biosciences - Botucatu-SP, Brazil.

²Federal University of Mato Grosso do Sul (UFMS), School of Veterinary Medicine and Animal Science - Campo Grande-MS, Brazil

³São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science - Botucatu-SP, Brazil

Fade and death of *Apis mellifera* colonies are increasing worldwide. One of the main causes is the indiscriminate use of pesticides in crops, being the herbicides the most used nowadays, such as glyphosate and 2,4-D, which are often used combined. This study aimed to evaluate the behavioral changes of *A. mellifera* foragers exposed to these combined herbicides. The mortality of bees exposed by ingestion to different doses of both herbicides was analyzed for 24 hours to determine the lethal dose (LD₅₀): 273.93 µg/bee for glyphosate and 127.70 µg/bee for 2,4-D. After that, the sublethal (SD - 1/50 LD₅₀) and the combination doses were defined. Bees motor activity was evaluated through a behavioral observation box, in which the travel time of exposed bees was evaluated. The 4h lethal and 24h sublethal doses of the combined herbicides promoted locomotor alterations, decreasing or paralyzing bees, except for the 4h sublethal dose. Our study shows that high doses or prolonged exposure to combined herbicides can affect the foraging behavior and, consequently, the survival of the colony since it depends on the food resources collected by foragers.

Keywords: pesticides, toxicity, behavioral changes.



Social stress increases anxiety-like behavior equally in male and female zebrafish

Brenno Bozi^{1*}, Jeane Rodrigues², Monica Lima-Maximino³, Diógenes Henrique de Siqueira⁴, Marta Candeias Soares⁵, Caio Maximino¹

1 Faculdade de Psicologia, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará

2 Programa de Pós-Graduação em Reprodução Animal da Amazônia, ReproAmazon – Universidade Federal do Pará

3 Departamento de Morfologia e Ciências Fisiológicas, Universidade do Estado do Pará, Campus VIII

4 Faculdade de Ciências Biológicas, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará

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Zebrafish anxiety-like behavior was assessed in the novel tank test after the formation of dominant-subordinate hierarchies. 10 pairs of animals were subjected to dyadic interactions for 5 days. After this period, a clear dominance hierarchy was established across all dyads, irrespective of sex. Significant effect of social status on bottom-dwelling ($Z = -3.4641$, $p = 0.0005$), time on top was also observed ($Z = 5.1656$, $p < 0.0001$), absolute turn angle ($Z = -4.5975$, $p < 0.0001$), and freezing ($Z = -4.7231$, $p < 0.0001$) were observed with both male and female subordinates showing more bottom-dwelling, absolute turn angle, and freezing than dominant animals. Finally, no effect of status was observed in swimming speed ($Z = -0.47524$, $p = 0.6346$). The results suggest that subordinate males and females have higher anxiety.



Effect of ocean warming on fototaxis and buoyancy activities of seabream larva

Mai M^{1*}, Pegado MR¹, Caramelo J¹, Mendes AC², Pousão-Ferreira P², Rosa R¹, Pimentel M¹

¹MARE - Marine and Environmental Sciences Centre, Faculdade de Ciências da Universidade de Lisboa, Av. Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal

²Instituto Português do Mar e da Atmosfera, Av. 5 de Outubro, 8700-305 Olhão, Portugal

Oceans are suffering dramatic changes such as ocean warming. According to last IPCC report, in a business-as-usual scenario, the temperature is expected to increase 4°C by 2010. Even that ocean warming effects on fish early ontogenetic stages have been deeply studied, there is still a limited understanding on the effect of transgenerational adaptation to climate-change related conditions. Most studies only involve a single life-stage, neglecting the potential for acclimation that in long-terms can be an important mechanism to mediate impacts on marine species. Therefore, the present work aimed to investigate if parental exposure of seabream (*Sparus aurata*) to ocean warming (+Δ4 °C) affects the progeny's early behaviour. To unravel these effects, we collected eggs from parents kept for 5 months in a control temperature (16°C) and others in warming conditions (20°C). In a multi-factorial design, we assigned the progeny to both treatments. Subsequently, we analysed larva with 5 and 10 DPH submitted to fototatic and buoyancy trials and recorded their behaviour after an acclimation period. We will then present preliminary data concerning how parental acclimation might affect the impact of warming on these behaviour trials during the early development of seabream.



Parturition of a captive Brazilian dwarf deer (*Mazama nana*)

Valdir Nogueira Neto^{1*}, Eluzai Dinai Pinto Sandoval¹, Maria Helena Mazzoni Baldini¹, Mateus J. R. Paranhos da Costa², José Mauricio Barbanti Duarte¹

¹Deer Research and Conservation Center (NUPECCE). São Paulo State University (UNESP–FCAV) Jaboticabal, São Paulo/Brazil.

²UNESP, São Paulo State University, Faculty of Agricultural and Veterinary Sciences, Research Group in Ethology and Animal Ecology (ETCO Group), Jaboticabal, São Paulo/Brazil

The Brazilian dwarf brocket (*Mazama nana*) is the smallest red brocket of South America that is endangered by hunting and human activities. Thus, observation in the wild is difficult and captivity allows us to access behavioral information to improve management conditions of the species. We recorded one primiparous *M. nana* female aiming to describe one-hour antepartum behavior. During this time, we observed the dilation of the cervix characterized in three categories: attempt (arched position of the back, tail lifted, slightly flexed hind legs and contraction of the abdomen), walking (more than two steps) and contraction (lying down, strong contraction of the abdomen). One hour before the fawn delivery, we observed 11 contraction series, with an average time of 394.7 seconds (± 220.5 seconds) between series with 26 expulsion attempts and 25 walking through the stall. As expected, contractions were more intense and frequent near the time of birth, the female remained lying down and fawning occurred quickly with a duration of 1 minute. This is the first description of parturition of *M. nana* in captivity
Keywords: Cervids, ex-situ management, reproduction.



Impact of anthropogenic noise on the behavior of meagre, *Argyrosomus regius*, juveniles

Rita Trábulo^{1,2}, André B. Matos¹; Manuel Vieira ^{1,3}; Ana Faria¹; Paulo J. Fonseca³; E M. Clara P. Amorim^{1,2}

1. MARE – Marine and Environmental Sciences Centre, ISPA-Instituto Universitário, Lisbon, Portugal.
2. Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal
3. Departamento de Biologia Animal and cE3c_Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

Meagre (*Argyrosomus regius*) is vocal fish that uses the Tagus estuary as reproduction grounds, an area with intense marine traffic. Here, we studied the effects of boat noise exposure on the behaviour of meagre juveniles, namely approach and pursuit. We exposed juvenile groups to either noise (boat noise playback) or control treatments (coils producing a similar electric field to the speakers) and recorded their pre- and post-feeding behaviour after 2 and 7 days of exposure. The ratio of approaches between post- and prefeed periods was always >1 in both groups, suggesting feeding arousal or increased food search. Interestingly, approach ratio decreased from day 2 to day 7 in the noise group but increased in the control suggesting that the increase in arousal or search behaviour with feeding is being affected by prolonged noise exposure. Pursuit was not affected. Whether these results have implications later in their development remains to be investigated. Also, tested fish were raised in an aquaculture facility, i.e. in much noisier environment than the natural one. Further studies with wild meagre could help shed some light on this issue and will give us a better understanding of how anthropogenic noise can impact meagre early in life.



Spatial learning in old fish: environment enrichment associated with exercises do not improve the performance in zebrafish

Isabela Inforzato Guermandi¹ * , Bruno Camargo dos Santos²; Marina Sanson Bellot², Percília Cardoso Giaquinto³

1 General and Applied Biology postgraduate program, São Paulo State University/UNESP, Botucatu, SP, Brazil

2 CAUNESP – Aquaculture Center, São Paulo State University, Jaboticabal, SP, Brazil

3 Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, São Paulo State University, SP, Brazil

Environmental enrichment (EE) and the exercise's neuromodulator potential emerge as promising strategies to increase connectivity between cells from brain areas affected by the neurodegenerative processes. We investigated the effects of EE with intense physical activities on memory and spatial learning in old zebrafish (*Danio rerio*). Four treatments were proposed: 1) EE and exercise, 2) EE, 3) exercise, 4) sedentary and monotony. All fish were trained in 3 different positions (3 repetitions/position) to find a rewarding shoal in a maze before the probe. We evaluated the latency to interact with the shoal (performance), latency to make a wrong choice (staying on the route) and total number of errors (exploitation) of fish. Performance, permanence on the route and exploration were not influenced by exercise, EE or its association in old zebrafish. Intense exercises in old individuals can act as a metabolic stressor with deleterious effects. New environments, as the maze, improved the spatial learning in sedentary and monotony fish. Even for a short period, to break monotony has a strong impact for individuals totally deprived of other stimuli. We conclude that structural enrichments associated with intense and continuous exercise do not improve memory and spatial learning in old zebrafish.

Key words: translational medicine, animal navigation, cognition



Participation Of The 5-HT_{2C} Receptor In Conditional Approach In Zebrafish (*Danio rerio*)

Ana Flávia Pimentel, Caio Maximino

Faculdade de Psicologia, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará

Conditional approach is a cooperative strategy for predator inspection, has serotonergic modulation in guppies, and has also been demonstrated in zebrafish. The role of the 5-HT_{2C} receptor was evaluated in this behavior by treating adult zebrafish (n = 21/group) with the agonist MK-212 and presenting an animated predator stimulus (*Nandus nandus*) in an aquarium where a mirror was in parallel, simulating a conspecific that mimics all the actions of the focal animal. Activation of the 5HT_{2C} receptor by MK212 decreasing predator inspection ($t_{[gl = 24.978]} = 2.679$, $p = 0.013$), but not freezing ($\chi^2 = 1.9611$, $p = 0, 16139$) nor erratic swimming ($\chi^2 = 1.0868$, $p = 0.2972$). These results suggest the participation of the 5-HT_{2C} receptor in conditional approach, which is not mediated by fear.



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Cannabidiol as a promising tool to increase fish welfare

Bruno Camargo-dos-Santos^{1,2*}, Marina Sanson Bellot^{1,2}, Isabela Inforzato Guermandi¹, João Favero-Neto^{1,2}, Renato Filev³, Eliane Gonçalves-de-Freitas^{2,4}, Rafael Henrique Nóbrega⁵, Percília Cardoso Giaquinto^{1,2}

¹ Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, São Paulo State University, SP, Brazil.

² CAUNESP – Aquaculture Center, São Paulo State University, SP, Brazil.

³ Department of Psychiatry, Escola Paulista de Medicina, Universidade Federal de São Paulo, SP, Brazil. ⁴ Departamento de Zoologia e Botânica, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, SP, Brazil.

⁵ Reproductive and Molecular Biology Group, Institute of Biosciences, São Paulo State University, Botucatu, SP, Brazil.

In aquaculture, investigating strategies that improve fish welfare, mitigating adverse effects of the rearing environment, it is of great importance. A substance that has potential to increase farming animals' welfare, is the cannabidiol (CBD). In some vertebrates, CBD presents anxiolytic properties, decreases aggressivity and stress, regulates reproduction, among other effects. Therefore, our aim is to verify the effect of different CBD doses (0,1,10 and 20mg/kg) in behavioral and morpho-physiological variables related to the welfare of Nile tilapia (*Oreochromis niloticus*), such as, aggressivity, stress and reproduction. Nile tilapia is an aggressive species and one of the most farmed fish worldwide, constituting a good study model. CBD was administered through diet to fishes (N=15) for 5 weeks. The 10mg/kg dose was efficient in decreasing fish's aggressivity over time, while the 20mg/kg dose attenuates the non-social stress, and both doses increased the gonadosomatic index. None CBD dose affected any feeding or growth variable, and consequently the meat production. So, CBD supplementation can bring benefits for animals' life and producers. Our study was the first to use the CBD as a tool to increase animal welfare, being promising in fish, and presenting a great potential to be explored in other farming animals.

Keywords: animal welfare, aquaculture, *Cannabis sativa*



Different housing conditions for zebrafish: what are the effects?

Priscila F Silva¹, Carlos Garcia de Leaniz², Fulvio A M Freire³, Vanessa A M Silveira¹, Ana C Luchiar¹

¹ Luchiar Lab, Physiology, Federal University of Rio Grande do Norte, Natal, Brazil

² Centre for Sustainable Aquatic Research (CSAR), Biosciences, Swansea University, Swansea, U.K.

³ Aquatic Fauna Lab, Botany and Zoology, Federal University of Rio Grande do Norte, Natal, Brazil

Zebrafish is a popular experimental model used worldwide. Still, some aspects of its husbandry are not well understood. Evidence has shown that housing conditions influence fish physiology and behaviour interfering on experimental results and leading to difficulties in data replicability. Here, AB zebrafish from two different origins and one wild-type zebrafish population were exposed to different light (74, 222 and 445 lx), background noise (39 and 62 dB), and temperature (25°C and 28°C) conditions. After seven days, fish behaviour was evaluated in the novel tank test. Our results demonstrated that temperature presented the most significant impact on fish response, increasing swimming velocity and total distance travelled. Increased light intensity altered fish behaviour, particularly freezing duration and distance from the tank bottom, indicating increased anxiety-like behaviour. Different levels of background noise did not cause significant changes in behaviour. Zebrafish strain populations also presented different responses: while AB fish was less affected by housing conditions, wild-type fish was highly affected by temperature, light, and noise variations. We suggest that holding conditions and fish origin should be more carefully considered and detailed described in the experimental design to avoid interference in data interpretation.



Long duration anaesthesia in zebrafish larvae: efficacy and behavioural effects

Sara Jorgea, b, c *, Ana Claroa, b, d and Ana M. Valentima,b

- a) Laboratory Animal Science group, IBMC – Instituto de Biologia Molecular e Celular, UP, Porto
- b) i3S – Instituto de Investigação e Inovação em Saúde, UP, Porto
- c) CITAB – Centro de Investigação e de Tecnologias Agroambientais e Biológicas, UTAD, Vila Real
- d) Faculdade de Ciências da Universidade do Porto

Zebrafish larvae are often used for developmental studies where immobility for imaging is attained for long periods using anaesthesia. However, long duration anaesthesia increases the risk of physiological and behavioural alterations, and overdose/mortality. Thus, this study evaluated the quality of long duration anaesthesia (15 h) and recovery of zebrafish larvae exposed to different anaesthetics. Six days post-fertilization (dpf) AB zebrafish larvae (n=25) were randomly assigned to: control (water), 2mg/L etomidate (ETO), 80mg/L MS222, 30mg/L clove oil (CO), 60+60mg/L MS222+isoflurane (MS222/ISO) and 5+50mg/l propofol+lidocaine (P/L) groups. The latency to anaesthesia induction and recovery were measured by the loss and regain of response to tail touch. At 26 and 50hours post-anaesthesia, larvae activity and anxiety-like behaviours were assessed. Our findings showed similar latencies to anaesthesia induction, but P/L animals presented the slowest recovery ($p<0.001$) compared to the other groups, except CO; while etomidate induced the quickest recovery ($p<0.001$), except compared with MS222/ISO. Nevertheless, ETO exhibited signs of light anaesthesia. Activity and anxiety-like behaviours (thigmotaxis) were not affected by the anaesthetics used, behaving at control levels. In conclusion, all protocols tested are suitable for long duration anaesthesia in 6 dpf zebrafish larvae, but etomidate protocol may not be suitable to sustain long-term immobility.



Acid-sensing ion channel blocker modulates behavioral deficits in a zebrafish based pain model

Isaac A. Adedara ^{a,b,c,*}, Fabiano V. Costaa,^b, Julia Canziana,^b, Eduarda Biasuza, Ebenezer O Farombic, Denis B. Rosemberga,^{b,d,*} *a Laboratory of*

Experimental Neuropsychobiology, Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. b Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. c Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria. d The International Zebrafish Neuroscience Research Consortium (ZNRC), 309 Palmer Court, Slidell, LA 70458, USA.

Acid-sensing ion channels (ASICs) is acknowledged to be associated with several neurological and pathological disorders. However, there is paucity of scientific information on the role of ASICs in the regulation of pain-related behaviors. The current investigation aimed to clarify whether amiloride, a nonselective ASICs blocker, modulates behavioral responses related to pain in a zebrafish-based pain model. At the outset we demonstrated that intraperitoneal injection (i.p.) of 0.25, 0.5, 1.0, and 2.0 mg/mL amiloride per se or vehicle did not alter zebrafish behavior in comparison with saline-treated fish 15 minutes after injection. Administration of 2.5% acetic acid (i.p.) occasioned writhing-like behavior evidenced by aberrant body curvature and reduced locomotion and motor activity. Lower amiloride doses (0.25 and 0.5 mg/mL) elicited attenuating effect, whereas 1.0 and 2.0 mg/mL abolished the pain-like responses. The protective influence of the highest amiloride dose investigated was associated with diminished writhing-like responses and enhanced locomotion and vertical activity. Taken together, amiloride antagonized behavioral impairments in a zebrafish-based pain model, substantiating the role of ASICs in modulating pain-related behaviors. Additionally, these data highlight the growing utility of zebrafish as a valuable model system in translational pain research and screening of new drug therapies.

Keywords: acid-sensing ion channels; amiloride; pain; behavior; zebrafish



Use of the water column by reef fish of different color patterns

Luísa Eduarda Fernandes dos Anjos¹, Eduardo Bessa Pereira da Silva^{2*}, Felipe M. Gawryszewski³

¹ Programa de Pós-graduação em Ecologia, Universidade de Brasília. Brasília, DF, Brazil.

²Ciências da Vida e da Terra, Faculdade de Planaltina, Universidade de Brasília. Área Universitária 1, Bairro Nossa Senhora de Fátima, Planaltina 73345-010, Brasília, DF, Brazil.

³Departamento de Zoologia, Universidade de Brasília. Campus Universitário Darcy Ribeiro, Asa Norte 70910-000, Brasília, DF, Brazil.

Color in animals responds to selective pressures and mediates an organism's relationship with the environment, and may play an important role in camouflage. Reef fish are the vertebrates with the greatest variety of pigment cell types known, and are important animals to understand how camouflage works and to better understand life in coral reefs. Thus, the present work addresses the use of the water column by reef fish of different color patterns. Here, we test the hypothesis that the mobility and stratum of the water column influence the color, predicting that sedentary animals that live very attached to the background take advantage of homochromy, that more mobile animals with demersal life apply the disruptive color while highly mobile pelagic species invest in silver bodies. For this we used data from 100 species of Brazilian reef fish available on the fishbase website. We observed that silver was more prevalent in the pelagic stratum, species with stripes occupied more frequently the demersal stratum and species with contrasting spots occurred mainly in the benthic stratum, confirming the raised hypothesis. The fish colors are well adapted to the occupation of the different levels of the water column, explaining in part the richness of color patterns existing among reef fish.

Key-words: coloration, camouflage, predation



***Hippopotamus* call active space estimation in an African habitat**

Paulo Fonseca¹, Julie Thévenet^{2,3}, Nicolas Grimault³, and Nicolas Mathevon^{2,4}

1 Departamento de Biologia Animal and cE3c—Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal. 2Equipe de Neuro-Ethologie Sensorielle ENES / CRNL, University of Saint-Etienne, CNRS, Inserm, Saint-Etienne, France. 3Equipe Cognition Auditive et Psychoacoustique CAP / CRNL, University Lyon 1, CNRS, Inserm, Lyon, France. 4Institut Universitaire de France.

Hippopotamus amphibius is an iconic African megaherbivore that spends daytime in groups protected from the sun in water bodies, grazing alone outside mostly at night. They exhibit a rich vocalizations' repertoire. The wheeze-honk, the most common loud vocalization, reaches a considerable distance in air, and may serve as a territorial vocalization, and to maintain contact during feeding time. We recorded wheeze-honk sounds both in air and water in an African lake habitat (Lake Chingute) in the Maputo Special Reserve, Mozambique. We estimate sound pressure levels reaching 95 – 109 dB re. 20 μ Pa at 1 m from a vocalizing animal. Simultaneous measurements of vocalizations at different distances (80 m to 1700 m), together with playback experiments, agreed with a geometrical spherical sound propagation model for hippo sounds. Considering day background sound level (45-50 dB), the active space of the hippo calls, estimated as the distance from a vocalizing male until attenuation reaches the background noise level, would be from 250 m to more than 1 km, likely more at the quieter nights. In contrast to general consensus, the low frequency wheeze-honk sounds attenuate underwater strongly with distance, and so could not serve distant underwater hippos acoustic communication.



Transgenerational effects of ocean warming on the behavior of sea bream (*Sparus aurata*)

Maria Rita Pegado^{1*}, Mai M1, Sampaio E1, 2, Caramelo J, Pousão-Ferreira P3, Mendes AC3, Rosa R1, Pimentel M1

¹MARE - Marine and Environmental Sciences Centre, Faculdade de Ciências da Universidade de Lisboa, Av. Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal ²Department of Collective Behaviour, Max Planck Institute for Ornithology, Konstanz, Germany ³Instituto Português do Mar e da Atmosfera, Av. 5 de Outubro, 8700-305 Olhão, Portugal

Sea surface temperature is expected to rise 4 °C by the end of this century in the IPCC business-as-usual scenario. Although the effects of ocean warming on fish behavior are already well-documented, it is still unknown whether these effects prevail across generations. The present work aimed to understand if parental exposure of sea bream (*Sparus aurata*) to ocean warming affected the progeny's behavior. Briefly, eggs collected from progenitors in control (16 °C, C) and warming (20 °C, W) were divided in a multi-factorial design, into control (CC and WC) and warming conditions (CW and WW). Subsequently, 10 days after hatching, the following behaviors were observed: time spent swimming, feeding (number of attacks, captures and failed captures), and resting (vertical position). Our results suggest that, in general, parental exposure may not affect larval behavior. Yet, while maintaining larva in the same treatment as the progenitors led to similar activity in both treatments (CC and WW), significant differences on the swimming activity were only observed between WC and CW. This suggests that changing the environment across one generation may lead to behavioral changes, although there is still no evidence of a transgenerational acclimation.



Hatching time and behavioral traits in zebrafish

Maria Elisa Leite-Ferreira, Bruno William Fernandes-Silva, Ana C. Luchiari.

Department of Physiology and Behavior, Bioscience Center, Federal University of Rio Grande do Norte, Natal, RN, Brazil.

Individuals within the same population differ in several ways. In previous studies, we observed that in fish, individuals that hatched earlier show more active behavior and are more exploratory than those hatched later, a characteristic of behavioral syndrome. One of the aspects relevant to this theory is the consistency of the behavioral differences between contexts and over time. Therefore, this study aimed at evaluating the relationship between time of incubation and behavioral consistency in two ontogenetic stages and in different contexts in zebrafish (*Danio rerio*). To establish individual differences, larvae were separated into 2 categories according to hatching time: early emerging (EE) and late emerging (LE), and tested in a risk-taking context (light x dark tank) at the 30th day post fertilization (dpf) and at the 120th dpf. Animals were also tested in a novel tank and novelty assessment contexts at 120 dpf. In the risk-taking test, EE animals presented shorter latency to take risks and stayed longer in the risky area compared to LE animals both at 30 and at 120dpf, indicating behavioral consistency over the ontogenetic development. In the novel tank and novelty assessment tests, the EE individuals were more exploratory and showed less anxiety-like behavior than the LE, suggesting behavioral consistency across context for both profiles. In general, the results obtained suggest that time of incubation is a reliable indicator of behavioral profile in zebrafish and behavior expressed by the two profiles keep consistency in time and contexts, thus corroborating the pace-of-life theory.

Keyword: *Danio rerio*, ontogenetic stages, behavioral profile, risk-taking.



Temporal and contextual consistency of individual differences in zebrafish (*Danio rerio*)

João Paulo Medeiros Mamede^{1*}, Heloysa Araujo-Silva¹, William Norton², Ana Carolina Luchiari¹

¹Departament of Physiology and Behavior, Bioscience Center, Federal University of Rio Grande do Norte, Natal, Brazil

² **Department of Neuroscience, Psychology and Behaviour**, University of Leicester, UK

The behavioral phenotype of individuals in a population presents some variations related to each one's life history and genetic background. Among the most studied behaviors, the bold and shy axis stands out, a dimension of behavioral variation related to an animal's propensity to take risks. However, animals' categorization and behavioral consistency have been of complex assessment. Here, we propose a method to categorize boldness in zebrafish and test its consistency. A light-dark emergency test was applied three times eliminating intermediate profiles. Then, very bold and very shy profiles were designated. The two groups were tested in the novel tank, open field, social preference, and mirror test to evaluate context consistency. Fish were also tested 2 times, with an interval of 30 days to evaluate temporal consistency. Bold animals showed less anxiety-like behavior when presented to novelty and exploratory contexts, decrease attachment to the shoal and increased aggressive response. Shy animals presented opposite responses. The behavioral manifestations were closely related two times fish were tested. Overall, we present a robust method to characterize behavioral profile in zebrafish, and the results showed that in most contexts, the individual differences maintain consistency, even when tested with a time interval.



Exploring ethology as a novel approach to examine the distribution of the endangered European eel in Madeira Island

Inês Órfão¹, Isabel Domingos^{2,3}, Ricardo Rocha⁴ and João Canning-Clode^{1,5}

1 MARE – Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Madeira Island, Portugal 2 MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, 3 Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, 4 CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal, 5 Smithsonian Environmental Research Center, Edgewater, USA

The European eel (*Anguilla anguilla*) is a Critically Endangered (IUCN) fish with nocturnal and benthic habits. The distribution of the species has been long investigated across Europe and North Africa mainly recurring to capture methods. However, little is known about its distribution in the Atlantic islands of Macaronesia. In this context, we examine for the first time the eel population inhabiting Madeira Island and test a new non-invasive sampling method to study this species distribution. As eels can apparently be more easily observed in this insular ecosystem than throughout mainland, we record the occurrence and abundance of eels during 4 x 50 m linear transects and *ad libitum* sampling along selected streams. Simultaneously, we record abiotic and biotic variables, such as stream depth and presence of other species, respectively. This study will contribute to a better understanding of the distribution of the species throughout the island, as well as identify the most favourable environmental conditions. Ultimately, our results will provide key insights into the natural history and distribution of the European eel in Macaronesia and, therefore, contribute to the conservation of the species.



Sex Differences in Emotional Facial Expression Recognition

Thays Machado da Cruz^{1*}, Vinícius Betzel Koehler², Rosana Suemi Tokumaru³

¹ Psychology, UFES

² Psychology, Neuroscience & Behavior, McMaster University

³Professor, Departamento de Psicologia Social e do Desenvolvimento, UFES

There are controversial results about the influence of sex on the recognition of expressions of emotion. Adults (n = 972, 59,1% females) were asked to correctly identify the emotion in 26 computerized faces, 50% male, expressing happiness, fear, disgust, anger, sadness and surprise with intensities of 30% and 70% and neutral expression. Surprise and happiness were the most correctly identified expressions, followed by anger, sadness, disgust and fear, regardless the gender of the participant. Participants also made more correct identifications of anger, disgust, sadness and neutral on male faces and happiness on female faces. Women made more correct identifications of anger 70% on male faces and happiness and neutral on female faces. We concluded that sender's and receiver's sex influence the recognition of expressions. Our results confirms findings indicating that recognition depends on the intensity and type of emotion. Overall the results revealed few sex differences in emotional expression recognition that may be linked to the social regulation of emotions. These results indicate the possibility that men and women were subjected to similar selective pressures along evolution of emotional recognition and that sex differences observed in other studies might be due to the social regulation of emotional expression.

Key-words: Social Cognition; Emotional Facial Expression; FaceGen Modeller



Open-source behavioural apparatus for memory assessment for the AB and TU zebrafish strain

Jorge Ferreira^{1,2,3,4*}, Joana Silva^{1,2,4}, Sofia Barros^{1,2,4}, Inês Caetano^{1,2,3}, Pedro Fernandes⁵, Ana M. Valentim^{1,2}

¹i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

²IBMC - Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Portugal

³Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto

⁴Faculdade de Ciências da Universidade do Porto (FCUP), Universidade do Porto

⁵Faculdade de Engenharia da Universidade do Porto (FEUP), Universidade do Porto

Open source validated tools are important for affordable high-quality science. Zebrafish model is often used in behavioural sciences, thus validated tools must be developed for this species. We aimed to create an effective learning apparatus, custom-built using 3D printing technology, and custom-made hardware and software through the microcontroller technology decreasing costs and increasing flexibility. Here, a mild electric shock ($3.3 \pm 0.3V$ and $2A$ for $5sec$) is used as an aversive stimulus for the one-trial inhibitory avoidance task based on classical conditioning in 72 monthsold zebrafish. The use of this stimulus causes a robust and long-lasting memory learned in one session. The apparatus consisted in one aquarium with two compartments (white and black) where, during the conditioning session the shock was delivered in the preferred side (black), followed by immersion in MK-801, a compound known for inducing amnesia (positive control, $n= p \leq 1$), or in clean water (control, $n= p \leq 0.039$). Both the AB and the TU strain from the control group showed avoidance for the black side after conditioning, while MK-801 impaired memory, as latency to black compartment was not altered. Summing, this open-source apparatus is an affordable and validated option for memory assessment for the two most used strains of zebrafish.



Pronounced personality differences within and among colonies of a social vole

Rita S. Andrade^{1,2,*}, Sophie Von Merten^{1,2}, M^a Luz Mathias^{1,2}, Susana A. M. Varela^{3,4}, Ana M. Cerveira^{1,5}

1 CESAM – Centro de Estudos do Ambiente e do Mar, Aveiro 2 Faculdade de Ciências da Universidade de Lisboa 3 Instituto Gulbenkian de Ciência, Oeiras, Portugal 4 ISPA – Instituto Universitário, Lisboa, Portugal 5 Departamento de Biologia, Universidade de Aveiro

Highly social species live in groups composed by many individuals. The array of behavioural traits displayed by the different elements of the group can play a key role in the dynamics, survival, and fitness of the colony as a whole. Having different individual personalities within a group can be advantageous, allowing for task division and specialisation. Having to dig and maintain a complex system of underground holes and tunnels, foraging for food on the surface, and helping to care for the young, the Lusitanian pine vole, *Microtus lusitanicus*, is the perfect candidate to understand the interplay between each individual's personality and that of the group. In this study, we performed repeated tests of boldness and activity in individuals from laboratory colonies of wild Lusitanian pine voles. By testing across different generations, we examined how an individual's personality develops over time, but also how the personality composition of each colony influences collective behaviour in cooperative tasks. Our results show that individuals within colonies have different personality types, with high repeatability. Additionally, each colony also has a different composition of individual personality types. These results agree with the social niche specialization hypothesis that highly social species should have well pronounced personality differences.



Small seedeater passerine can learn from observing others. A test on serins (*Serinus serinus*) ability to learn from observing the foraging behavior of others
Ricardo Silva, Paulo G. Mota

Departamento Ciências da Vida, Universidade de Coimbra CIBIO

Social learning in birds is documented for only a limited number of species, including Great tits, but not for other small passerines. We wanted to test the cognitive abilities of individuals from less social, granivorous species for the possibility of social learning. We used a small social granivorous passerine, the Serin (*Serinus serinus*), on a foraging association task. We trained birds to feed on hidden holes covered with a colour disk, and used them as demonstrators. About half of individuals, both males and females, learnt to perform the task after observing the demonstrators, showing that they are capable of social learning. Memory retention ability was assessed 15 days later, with a success rate of 100%. Subject to a reversal learning association task, about half of the learners were capable of switching for the new choice colour. Some intriguing interaction between sex of demonstrator and sex of learners are discussed.



Aggression in the presence of opponents, rather than towards them: a safe strategy to manage dominance hierarchies?

Patrícia Beltrão, Ana Cristina R Gomes, Gonçalo C Cardoso,

CIBIO/InBIO—Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

Group-living animals often form dominance hierarchies, which then influence aggressive behaviours towards others. Knowing how social dominance hierarchies are established and maintained is critical for understanding social structure and the cognitive abilities of individuals. Here we studied patterns of social aggression in common waxbills (*Estrilda astrild*) living in a large mesocosm, namely how they behave towards potential opponents: individuals similar in dominance rank to themselves, and distant individuals in the social network. We found that waxbill aggression rates did not differ depending on the similarity in dominance rank or the social proximity of opponents. Instead, aggression rates of focal waxbills were higher when the audience included bystanders of similar dominance rank to the focal waxbills, or bystanders socially-distant to the focal waxbills. We suggest that showing off aggressiveness in the presence of potential opponents, rather directly attacking those opponents, may be a strategy to manage dominance hierarchies that avoids potential costs (e.g., retaliation, injury) of engaging in a direct fight with those opponents.



Looking at others' faces may be more important in early infancy

Natalia Albuquerque, Juliana França, Patrícia Izar

Institute of Psychology, University of Sao Paulo, Sao Paulo, Brazil

Living in social groups confers individuals many advantages, one being the possibility to obtain relevant information from others. We investigated the opportunities to obtain visual information from the face of others, which we call VAF (Visual Access to Faces) in a wild population of capuchin monkeys from Brazil. We looked at four infants, aged two and nine months old and tested the hypothesis that both the identity of the individual the infants were watching and the infants' age (2nd or 9th month) influenced the duration of these opportunities to obtaining information from others' faces. We looked at 323 VAF events (mean=2.539±0.489) using a GLMM (infants as a random factor). Identity of the other monkey was not significant, neither its interaction with month. However, month alone was significant ($p=0.016$), with a higher mean for the 2nd (3.296±0.653) than the 9th (1.415±0.560) month. From these results it appears that whom the infant is looking at is not relevant, however when, if earlier or later in infancy, is critical for the acquisition of this sort of social information. Cognitive development, cognitive biases and the differential relevance of the information acquired may be explanations to these findings.

Keywords: *Sapajus libidinosus*, social behaviour, social cognition, social regulation, visual processing

Funding: Fapesp



Why are females ornamented? Plumage dichromatism explains female but not male territorial responses

Gabriel Macedo^{1,2}, Gustavo A. Bravo³, Rafael S. Marcondes⁴, Elizabeth P. Derryberry⁵, Cibele Biondo¹

1. Universidade Federal do ABC, São Bernardo do Campo, SP 09606-045, Brazil. 2. gabriel.biologia17@gmail.com, author presenting the communication. 3. Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA. 4. Museum of Natural History, Louisiana State University, Baton Rouge, LA 70803, USA. 5. University of Tennessee, Knoxville, TN 37996, USA.

The importance of social selection in promoting the evolution of ornamentation in both sexes is poorly understood. We addressed this issue in antbirds (Thamnophilidae), a radiation wherein eumelanic plumage patches have evolved in both sexes. In antbirds, the degree of sexual dichromatism differs among closely related species due to eumelanic patches in either sex. If eumelanic patches evolved by social selection, individuals of species bearing more conspicuous eumelanic patches should display stronger territorial responses regardless of their sex. We simulated territorial intrusions in the field and measured responses by social pairs of three sister species pairs: two differing in female coloration and one differing in male coloration. Females of species bearing more conspicuous eumelanic patches responded more strongly than females of species bearing less conspicuous eumelanic patches. However, males responded similarly regardless of interspecific coloration differences. Our results suggest that greater female ornamentation may reflect stronger competition in ornamented females for territories and, possibly, mates. In species pairs that differ in male ornamentation, female mate choice, and not territorial competition, may drive the evolution of male ornamentation. Our study highlights that social selection may drive the evolution of ornamentation in both sexes, thus affecting sexual dichromatism.

Key words: social selection, sexual selection, sexual dichromatism, acoustic playback experiments, bird song, Neotropical Suboscines.



Collective behaviour and social dynamics during interspecific collaborative hunting between octopus (*Octopus cyanea*) and multiple fish species

Eduardo Sampaio^{1,2,3*}, Vivek Sridhar^{2,3,4}, Fritz Francisco^{5,6}, Maté Nagy^{7,8}, Paul Nührenberg^{2,3,4}, Iain D. Couzin^{2,3,4}, Rui Rosa¹, Simon Gingins^{2,3,4}

¹MARE - Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Lisboa. Portugal.

²Department of Collective Behaviour, Max Planck Institute of Animal Behavior, University of Konstanz, Germany.

³Centre for the Advanced Study of Collective Behaviour, University of Konstanz, Germany.

⁴Department of Biology, University of Konstanz, D-78547 Konstanz, Germany ⁵Technische Universität, Science of Intelligence (SCIOI), Marchstr. Berlin, Germany ⁶Humboldt University, Romanczuk Lab, ITB, Humboldt Universität zu Berlin, Germany ⁷MTA-ELTE "Lendület" Collective Behaviour Research Group, Hungarian Academy of Sciences, Pázmány P. stny. Hungary

⁸Department of Biological Physics, Eotvos Lorand University, Pazmany P. stny. Hungary

In collective behaviour, complex coordination and individual/group decision-making can emerge from simple, localized rules of interaction, considering every individual as identical. However, in biological systems, variation amongst individuals can alter the “weight” of specific individual’s decision within a group, creating a dynamic network of attraction-repulsion forces driving individual and group movement. Parallely, sociality has the potential to drive brain evolution and cognition, but the role of individual cognition on collective movement and group decision-making constitutes a largely unexplored field. Using SCUBA, we recorded interspecific hunting events between *Octopus cyanea* and multiple partners (i.e. various fish species), and obtained animals tracks over a 3D reconstructed habitats, which enables multi-scale analyses gauging novel and key parameters in interspecific collectives, e.g. movement synchrony between *O. cyanea* and fish partners; leader-follower dynamics; habitat, social, and individual level factors influencing leadership; and presence/absence of social rules. From individuals to collective, understanding the underpinnings of these interspecific interactions can potentially deepen our knowledge on cephalopod cognition in a social context, mechanisms of intentional/unintentional communication, and the influence of higher order traits (morphological, behavioural, and cognitive) on collective movement and decision-making in naturally-occurring heterogenous groups.



Functional specialization of social learning in *Drosophila melanogaster*

Carla Simões-Henriques¹, Joana Marcos¹, Élio Sucena¹, Maria L. Vasconcelos², Susana A. M. Varela^{1,3*}, Rui F. Oliveira^{1,2,3*}

1 – Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2 – Champalimaud Centre for the Unknown, Lisboa, Portugal; 3 – ISPA-Istituto Universitário

Environmental information can be social or asocial. Animals need cognitive abilities to handle with both types of information and their complexity. However, whether social and asocial information are processed by a single (general-purpose) or by distinct (special-purpose) cognitive mechanisms is still elusive. Answering to this question is cardinal to our understanding of how social cognition and sociality have evolved. To address this question, we studied oviposition-site choice aversive conditioning in fruit flies. We used 40 lines from a *Drosophila* panel, constituted of several isogenic sequenced lines that together represent the genetic variation of a natural population. We measured each line phenotypic variation for their social and asocial learning abilities. Then, we assessed the statistical association between the social and asocial learning phenotypes and the lines' genetic information. The analyses unveiled totally different sets of candidate genes for social and asocial learning, suggesting distinct cognitive mechanisms. To validate this finding, we are now using another *Drosophila* genetic toolbox, which consists of silencing each candidate gene at a time. So far, we have found genes that affect both learning phenotypes, but also cases of specialization. This, again, suggests that social learning probably underwent an evolutionary functional specialization distinct from asocial learning. The present abstract is submitted with preference for oral presentation.



Effects of early ethanol exposure on the behavior of adult zebrafish (*Danio rerio*) populations

Thaís Agues-Barbosa, Jackson Nazareno Gomes de Lima & Ana Carolina Luchiari

Department of Physiology & Behavior, Universidade Federal do Rio Grande do Norte, Rio Grande do Norte, Brazil

The Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE) is a condition that cause mild impairments in one's behavior as a result of early exposure to ethanol. Since genetic and physiological traits influence the response to ethanol, comparative studies among populations are useful. In this study, we evaluated how embryonic ethanol exposure affects the behavioral responses of AB, TU, and WT zebrafish populations at the adult stage. Embryos at 24hpf were exposed to 0% or 1.0% ethanol for 2h. Fish were tested for locomotor and anxious-like behaviors in a novel tank at the adult stage (90dpf). We observed that both AB and TU exposed to 1.0% embryonic ethanol exhibited hyperlocomotion (increased speed and distance traveled) and anxiogenic response (decreased distance from the bottom), while WT fish showed unaltered responses. For the first time, zebrafish populations in later ontogenetic stages were shown to present different behavioral responses to embryonic ethanol exposure. Our results suggest that AB and TU fish show similar and robust responses, while the WT population shows higher variability. These data support that inbred populations (AB and TU) suit better in translational studies, as it offers reliable results, in contrast to outbred populations (WT) that display highly variable genomes.

Keywords: alcohol; anxiety-like; motor response; inbred populations; outbred populations.



Individual differences and effects of alcohol in the neurophysiology of zebrafish (*Danio rerio*)

Heloyza Araujo-Silva¹, Maria Elisa Leite-Ferreira¹, Waldo Lucas Luz², Danielle Valente Braga², Anderson Manoel Herculano², Eduardo Pacheco Rico³ and Ana Carolina Luchiari¹

¹Department of Physiology and Behavior, Bioscience Center, Federal University of Rio Grande do Norte, Natal, Brazil ²Laboratory of Experimental Neuropharmacology, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil ³Laboratory of Experimental Neurology, Graduate Program in Health Sciences, University of Southern Santa Catarina, Criciúma, SC, Brazil

To survive environmental pressures, individuals within the same population outline coping strategies and develop behavioral resources according to the environment in which they evolved. To understand these differences, this study evaluated the effects of different alcoholic concentrations on the behavioral and neurophysiology profile of zebrafish (*Danio rerio*). Adult animals were separated into two behavioral profiles: bold and shy, according to the emergence order from a white to a black side of a shuttle box. Then animals were acutely exposed to alcohol treatments (0.00%, 0.10%, 0.25%, and 0.50%), and behavioral parameters were evaluated. Following alcoholization, animals were euthanized and the brains were subjected to High-Performance Liquid Chromatography analysis. The neurotransmitters adenosine, dopamine, GABA, and glutamate were quantified. Bold 0.00% animals showed higher speed while moving. The distance from the bottom tank was increased by a low dose (0.10%) in both profiles. Both alcohol concentrations and behavioral profiles were significantly affected glutamate, dopamine, and adenosine analyzes. Our results corroborate previous findings that alcohol differently affects the behavioral profiles, with bold animals apparently more resistant to these changes.



Behavioral effects following chronic exposure of the β -carboline alkaloid harmine, a main constituent of Ayahuasca, in zebrafish (*Danio rerio*) models of anxiety-like behavior and aversive memory

Hector Quinones Vargas^{1,2}, Ana Carolina Luchiari¹, Bruno Lobão Soares²

¹Department of Physiology and Behavior, Bioscience Center, Federal University of Rio Grande do Norte, Natal, Brazil ²Department of Biophysics and Pharmacology, Bioscience Center, Federal University of Rio Grande do Norte, Natal, Brazil

Depression and anxiety disorders are the most prevalent mental illnesses, affecting more than 500 million people worldwide. A particular psychedelic known as Ayahuasca, made from the combined infusion of *Banisteriopsis caapi*, rich in monoamine oxidase inhibitors (MAOi) known as Beta-carbolines, and *Psychotria viridis*, rich in N, Ndimethyltryptamine (DMT), has stood out as a new treatment option for such disorders. In this study, we evaluated the effects of different doses of one of the main betacarbolines found in Ayahuasca (harmine) on behavioral profiles associated with zebrafish (*Danio rerio*) models of anxiety-like behaviors and cognitive memory deficits. Adult zebrafish were exposed to 2 different harmine concentrations of 0.02 and 0.2 mg/L, plus vehicle (DMSO) for the control group. Several behavioral parameters were assessed with a Conditioned Place Aversion (CPA) task, which include swimming speed, freezing, latency to cross, and time spent on each side of the CPA tank. It was observed that the 0.2 mg/L harmine dose group showed a significant reduction in average speed, total distance, and freezing behaviors, which model anxious-type behaviors, in relation to the control group ($p < 0.05$ in all cases). The results suggest that chronic harmine treatment may have a therapeutic role in tasks that model anxiety-like behavior and aversive memory.



Dietary supplementation of tiger nut alters sexual behaviors and biochemical parameters relevant to erectile function in L-NAME challenged rats

Ayodeji A. Olabiyia,b,c,* , Maria V. Morschc, Ganiyu Oboh, Maria R. Schetingerc

a Department of Medical Biochemistry, Functional Foods and Nutraceuticals Unit, Afe Babalola University, P.M.B. 5454, Ado-Ekiti, Nigeria b Department of Biochemistry, Functional Foods and Nutraceuticals Unit, Federal University of Technology, P.M.B. 704, Akure 340001, Nigeria c Department of Biochemistry and Molecular Biology, Center of Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, RS 97105-900, Brazil

Tiger nuts (TG) have reportedly used for the treatment of erectile dysfunction(ED) in folk medicine without scientific basis. Hence, this study evaluated the effect of TG on ED by assessing sexual behavioral responses, biochemical parameters relevant to ED in male rats by nitric oxide synthase (NOS) inhibitor, Nw-nitro-L-arginine methyl ester hydrochloride (L-NAME) treatment. Male rats were divided into five groups(n=10): Control group;L-NAME/basal diet;L-NAME/Sildenafil citrate;diet supplemented processed TG(20%)/L-NAME;diet supplemented raw TG(20%)/L-NAME. L-NAME pre-treatment(40mg/kg/day) lasted for 14 days while the female rats were fed standard diet. Sexual behaviors(copulation tendency and anxiety) were carried out after which arginase, acetylcholinesterase (AChE) and adenosine deaminase (ADA) activities as well as nitric oxide levels (NO) in serum, brain and penile tissue were measured. LNAME caused a decreased in the sexual behaviors evaluated while this effect reversed significantly by supplemented diets containing TG. In addition, L-NAME increased arginase, AChE and ADA activities and reduced NO levels. However, dietary supplementation with TG reduced the activities of the enzymes and up regulated nitric oxide levels when compared to the control groups. These enhanced activities could be part of the mechanism by which TG exert aphrodisiac properties. Quercetin was the most active component of TG by HPLC finger printing.

Keywords: L-NAME; Sexual behavior; Arginase; Acetylcholinesterase; Adenosine deaminase; Nitric oxide; Tiger nut; Quercetin



Oxybenzone effects on locomotor and social behavior in zebrafish

Ana Luisa Pires Moreira, Ana Carolina Luchiari

Department of Physiology and Behavior, Biosciences Center, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil

Oxybenzone is one of the most used UV filters. Due to its bioaccumulation and action as an endocrine disruptor, its ecotoxicological effects have been investigated. Here, we approach the effects of oxybenzone exposure on locomotion, anxiety-like and social behavior in zebrafish. Fish were exposed to different Oxybenzone concentrations (10, 100 and 1000 $\mu\text{g L}^{-1}$) for 15 days and then tested in a novel tank and shoal preference test. Fish were individually placed in a tank, and behavior was recorded for 5min. Then, another tank containing 6 conspecifics (same size) was placed close to the focal fish tank but an opaque partition prevented fish from visual contact. Fish behavior was recorded for 5 min, the partition was removed, and the shoal response was recorded for another 5 min. Fish exposed to oxybenzone showed reduced locomotion, decreased anxiety-like behavior (time at the bottom zone of the tank), and less time near/interacting with the shoal. As zebrafish present anxiety-like behavior when exposed to novelty and are social animals to optimize foraging and avoid predation, we observed that oxybenzone negatively affects the animal's natural behavior. These results show that oxybenzone chronic exposure increases risk-taking and may affect animals' survival and fitness.



Mesotocin influence of waxbills' social behavior

Sandra Trigo, Paulo A. Silva, Gonçalo C. Cardoso, Marta C. Soares

CIBIO/InBIO—Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

The neuropeptide oxytocin (OT) has been crucially implicated in the regulation of sociality, having well conserved patterns across vertebrates. Non-mammalian homologs of OT exist in other vertebrates (isotocin for fish and mesotocin (MT) for amphibians, reptiles and birds), all of which play crucial roles in modulation of social and reproductive behavior. In this study, we exogenously manipulated the MTergic system in a highly social bird, the common waxbill (*Estrild astrild*), and tested effects on affiliative and aggressive behavior by performing competition over food test. We observed a sex-specific effect of OT antagonist (OTA) administration in aggressiveness, with a decrease in OTA treated females. Both OTA and MT treatment decreased affiliative behavior (allopreening), with the effect of MT treatment being stronger in males than in females. Birds treated with MT also fed less and were less active than controls. Our findings demonstrate for the first time, a sex-specific relevance by the MTergic system, in the modulation of male affiliation and female aggression pathways. Moreover, it is also involved in regulating activity and feeding. It is therefore possible that the anorexigenic effect of OT-like peptides is well conserved in vertebrates.



Role of cgmp in behavioral sensitization of anxiety-like responses in zebrafish (*Danio rerio*, Hamilton, 1822)

João Alphonse Apóstolo Heymbeeck¹, Wilker Leite do Nascimento¹, Tainá Pontes Brito Dias¹, Caio Maximino², Monica Gomes Lima-Maximino²

1. Laboratório de NeuroFarmacologia e Biofísica, Departamento de Morfologia e Ciências Fisiológicas – CCBS, Universidade do Estado do Pará – Campus VIII, Marabá, Pará, Brasil 2. Laboratório de Neurociências e Comportamento “Frederico Guilherme Graeff”, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Marabá, Pará, Brasil.

Based on evidence that supports the participation of the nitregric system in the consolidation of the stress response, the present work aimed evaluate the role of cGMP produced by the nitric oxide (NO) signaling pathway in the sensitization of anxiety-like responses observed in an animal model. 102 zebrafish of the longfin phenotype were treated with vehicle or a soluble guanylate cyclase enzyme inhibitor (ODQ; 3.33 mg/kg, i.p.) 30 or 90 minutes after exposure to water or the alarm substance (AS). Behavior was analyzed 24 hours after exposure using the light/dark preference test. The parameters considered were scototaxis, erratic swimming, risk assessment, thigmotaxis, freezing, trashing, duration of entries, and number of entries in the white side. 30 minutes after AS exposure, scototaxis [$F(1.83)=9.195$; $p=0.0032$] and erratic swimming [$F(1.83)=10.16$; $p=0.0020$] were sensitized, and treatment with ODQ reversed the effects on scototaxis [$F(1.83)=8.709$; $p=0.0041$], erratic swimming [$F(1.83)=4.234$; $p=0.0428$], and duration of entries [$F(1.83)=4.072$; $p=0.0468$]. At 90 minutes, there was an effect of treatment with ODQ on duration [$F(1.74)=7.103$; $p=0.0094$] and number of entries [$F(1.75)=14.47$; $p=0.0003$]. A participation of cGMP in scototaxis, erratic swimming, duration and number of entries on the white side is suggested.

Keywords: *Zebrafish*, behavioral sensitization, anxiety-like, nitric oxide.

Apoio financeiro: CNPq-Brazil.



***Acta Ethologica* (SPE's journal): developments and publication tips**

Peter K. McGregor

ISPA – Instituto Universitário,
Rua Jardim do Tabaco 34, 1149-041 Lisbon, Portugal

Launched in 1998 with ISPA as the society journal of the Portuguese Ethological Society (SPE), *acta ethologica* is published as three hard copy issues per year and online by Springer Nature. It is a significant journal within the field of behaviour, with a 5-year impact factor of 1.692 and 29,505 downloads in 2020. In this talk I will outline recent developments (e.g., the hybrid open access model and the adoption of STRANGE guidelines) and the rapidly changing aspects of journal publishing more generally that will affect *acta ethologica* including preprint servers, plagiarism checkers and grant bodies requiring open access publication. I will also highlight ways authors can maximise the chance that their ms will be accepted for publication (e.g., the information to include in a covering letter at submission, details required by the online submission process and how best to respond to referees' comments). Finally, if you have ever wanted to know why the journal title is in lower case letters, this talk is for you.